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GROWTH PARTICULARITIES OF AMERICAN POKEWEED – PLANT WITH MULTIPURPOSE UTILIZATION

Raisa IVANOVA[1*], Jana SIMKOVA[2], Jan BRINDZA[2]

[1]Institute of Genetics, Physiology and Plant Protection, Chisinau, Republic of Moldova
[2]Institute of Biodiversity Conservation and Biosafety, Slovak University of Agriculture in Nitra, Slovak Republic
*Corresponding author: ivanova_raisa @yahoo.com

ABSTRACT

American pokeweed (*Phytolacca americana* L.) is a good ornamental plant due to its ability to grow rapidly, broad and tall vegetation, and attractive grape-like fruits. However, American pokeweed and some useful chemical constituents, which accumulate in different parts of this plant, until present, in our opinion, are not utilised sufficient effectively. The leaves and seeds of *Phytolacca americana* L. produce pokeweed antiviral protein with increased antiviral and antifungal activities. Natural dyes accumulated in fruits can be used in the food, cosmetic, pharmaceutical and textile industries. The purpose of this work was to study the growth particularities of pokeweed plants propagated by seeds, and to establish the content and antioxidant activity of main constituents (polyphenols and colorant substances) in ripe fruits. American pokeweed demonstrated the excellent adaptivity of seeds and seedlings under different condition of germination, transplantation and cultivation. In season of 2019, the vegetation cycle of *Phytolacca americana* L. annual plants continued maximal 210 days (from March to November). The biometrical indexes of stems, racemes, fruits and seeds corresponded to similar characters reported by other scientists. The aqueous and hydroalcoholic extracts were obtained from ripe fresh fruits. The biggest amount of polyphenolic and colorant substances was found in aqueous extracts. The antioxidant activity of *Phytolacca americana* L. fruit extracts, evaluated *in vitro* by potentiometric procedure against the peroxyl free radicals, was in direct proportion to the content of polyphenols (r^2=0.9563) and colorant substances (r^2=0.9808). The colorant substances from American pokeweed fruits possessed the high antioxidant activity, IC_{50} was equal to 259.65±2.60mg/l.

Keywords: *American pokeweed, growth particularities, fruit, colorant substances.*

INTRODUCTION

American pokeweed (*Phytolacca americana* L.) is the herbaceous perennial plant with multipurpose utilisation. Due to annual renewal of the aerial part (plants resprout from a large fleshy taproot), rapid growth ability, and attractive grape-like fruits the American pokeweed is wide used as an ornamental plant in horticulture

and botanical gardens. American pokeweed berries are reported to be a good source of food for songbirds and other bird species and small animals. Young leaves and stems of American pokeweed in spring are harvested in order to prepare the different food of Native-American, African-American and Southern cuisines (Balogh and Juhasz, 2008). The extracts from aerial part and roots of *Phytolacca americana* L. exhibit various pharmacological effects (anti-inflammatory, antifungal, antiproliferative activity), which allowed the development and standardization some homeopathic preparations. The matrix tinctures derived from the fresh roots and recommended for treatment of mumps, arthritis and various skin diseases were included in the Homeopathic Pharmacopeia of Germany and Brasilia. The Chinese Pharmacopeia describes the powder from dried roots as an allopathic medicine for carbuncles treatment, as well as a purgative, salve, bronchodilator and diuretic. The pokeweed antiviral protein (PAP) produced in leaves and seeds of *Phytolacca americana* L. possesses an increased antiviral and antifungal activities (Tumer *et al.,* 1999; Patra *et al*., 2014; Domashevskiy and Goss, 2015). Purified PAP was found to be a potent inhibitor of eukaryotic protein synthesis and viruses of several plant and animal. The mechanism of its cytotoxicity, PAP-induced disease resistance in plants and application to agriculture were described in review (Di and Tuner, 2015). Fruits and natural dyes accumulated in *Phytolacca americana* L. fruits have been used since the 17th century as an ingredient for pickled vegetable and red wines to give the products astringency, spice taste and attractive colour (Balogh and Juhasz, 2008; Florea and Donea, 2010). Actually, the possibilities of application the natural dyes of American pokeweed for food and textile industries are intensive studied (Liu *et al.*, 2014: Mchedlishvili *et al.*, 2014; Park and Jung, 2014; Belhadj, 2017). Thus, the all organ of American pokeweed plants contains the biologically active substances, but this species of plant and some useful chemical constituents, in our opinion, are not utilised sufficient effectively. In the Republic of Moldova and Slovak Republic, the plants of American pokeweed are propagated only in botanical collections. These plants could be reproduced by seeds, sprouts from a large fleshy taproot and micropropagation using various organ cuttings of plant (Florea and Donea, 2010; El-Minisy *et al.*, 2017). The purpose of this work was to study the growth particularities of American pokeweed plants propagated by seeds, and to establish the content and antioxidant activity of main constituents (polyphenols and colorant substances) in ripe fruits.

MATERIALS AND METHODS

Seeds of *Phytolacca americana* L. were collected from plants growing on the territory of the Republic of Moldova (Chisinau) and the Slovak Republic (Nitra). A part of seeds was harvested in September in the end of seasons from fresh ripe fruits, and other part of seeds - in February from fruits dried and overwintered on the shrubs. The seeds were stored in the same conditions: at room temperature 18-20 ºC, in darkness. The morpho-biological features of *Phytolacca americana* L. plants were studied in the season of 2019. In the last decade of March 2019, the

seeds were sown for germination under different conditions: directly in the field, and on pallets in the greenhouse to obtain the seedlings. Planting density in the field was 80 cm between rows and 50 cm between plants. Field experiments were carried out at the research station of Institute of Genetics and Plant Protection in Chisinau area of Republic of Moldova (lat. 47°01', long. 28°75', alt. 85 m above sea level). The aqueous, ethyl alcoholic (96%) and hydroalcoholic (40%) extracts were obtained from ripe fresh fruits. The total polyphenolic content in extracts was appreciated by Folin-Ciocalteu method in equivalent to gallic acid (Singleton *et al.,* 1999). The concentration of colorant substances was determined using UV-Vis spectrophotometry at 520 nm. Radical scavenging activity of fruit extracts was detected *in vitro* by potentiometric procedure (Sano *et al*., 2003) in our modification (Ivanova, 2016), using 2,2'-azobis (2-amidinopropane) dihydrochloride as generator of reactive peroxyl ROO· radicals. Antioxidant activity were calculated from dose-activity curves and expressed as IC_{50} (concentration that inhibit 50% of free radicals) index of extracts.

RESULTS AND DISCUSSION

The first seedlings growing in the field conditions were observed after 55 days from sowing date. No differences in rate of germination between fresh collected and overwintered seeds was determined. It is necessary to note that in greenhouse conditions the seedlings appeared earlier than in the field for 10-15 days. Seedlings were represented by a small orthotropic shoot 2-3 cm long with 3-4 leaves and weakly expressed internodes. In the last decade of May, the seedlings from greenhouse were transferred in the field. Despite the fact that the various recommendations for cultivation of *P. americana* mentioned that the seedlings do not like transplantation, in our experiments transferred seedlings easily adapted to the new growing conditions. During 25-30 days after transferring, the young generative plants had one leafy orthotropic stem with length of 40-50 cm and 2-4 side branches (fig.1 b). The stems are straight, thick, juicy, green at beginning of growth and reddish as they grow older. The leaves were of different sizes (from 10 to 30 cm), which varied depending on the leaf position on the stem. The pokeweed leaves are green, oblong or ovate-lanceolate in shape, at the apex acute (fig.1 c). Damaged leaves exude a specific aroma.

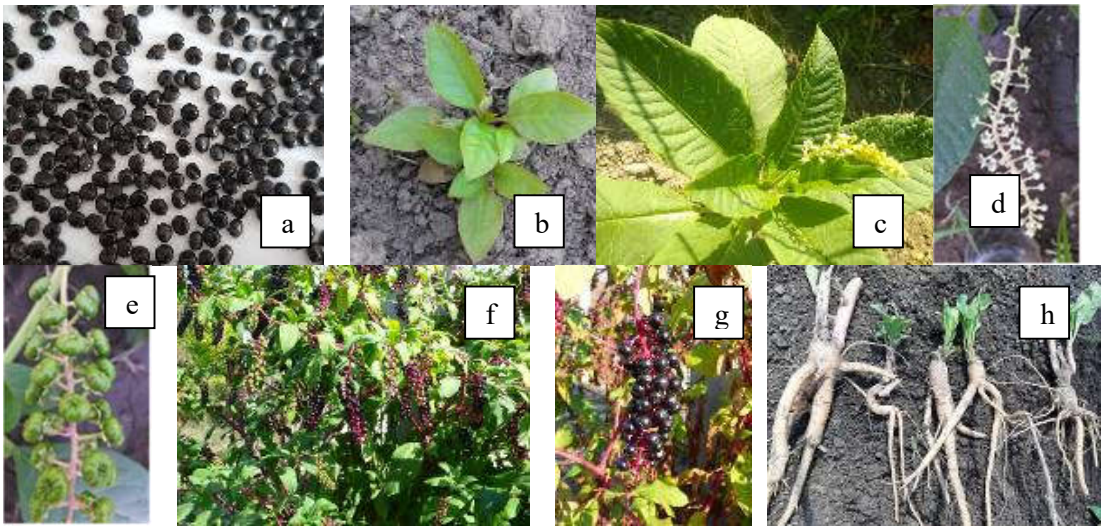

Figure 1. *Phytolacca americana* L.: a) seeds; b) seedlings; c) flowering plant; d) flower; e) raceme with green berries; f) plant in fruits ripening stage; g) ripe fruit; h) roots.

At the mid-June, the flowering stage began (fig.1 c), which lasted until the first decade of September. American pokeweed inflorescences are racemose, multi-flowered, more frequent 10-15cm long. The flowers are white, small (diameter 0.5 cm, length 2.5 cm), bisexual (fig.1d). Flower racemes was typically erect when in bloom but begin to droop as the fruit develops. A distinctive feature of *Phytolacca americana* L. from other species of *Phytolacca* is that the inflorescences are inclined under the heaviness of initially light green and then reddish-purple berries (fig.1e, f, g). Thus, already year-old American pokeweed plants bloomed in abundance and bore fruit. In our experiments the plants did not get sick and did not suffer from pests. Thus, in vegetation season of 2019 the annual plants of *Phytolacca americana* L. demonstrated the following duration of growth stages: 40-55 days for seeds germination, 25-35 days of active growth of stem, branch, and leaf areas, 55-60 days of flowering and 50-60 days of fruits ripening. Flowers and fruits were presented simultaneously at different stages of development. In general, the vegetation cycle persisted from 160 to 210 days, or from March to November. In pedoclimatic conditions of the Republic of Moldova, the seedlings developed in the soil a multi-headed short rhizome with a thick, fleshy and fusiform rod roots, from which the plants will resprout in spring of next years (fig.1 h).

Previous studies on the propagation of *Phytolacca americana* L. in the Republic of Moldova (Florea and Donea, 2010) led to the conclusion that there is a direct correlation between biometric characteristics, such as the height and mass of the generative stem, the number of leaves, inflorescences and fruits on it. The strongest correlation was revealed between the height of the generative stems and other studied characteristics of productivity. One generative stem of 1.2-2.6m tall had from 16 to 39 number of inflorescences and produced 696-2083 of berries (Florea

and Donea, 2010). Each berry contains about nine seeds; consequently, one plant of American pokeweed can produce 3,500-18,000 seeds annually. In order to develop these investigations, the length of American pokeweed racemes, weight of berries from one raceme, seeds size and 1000 seeds weight were determined. In 2019 season the length of racemes varied from 8.8cm to 17.5cm, 76.2% of racemes had the length in limits of 15.00±1.85cm. The diameter of American pokeweed berries was 8.73±0.25mm. Weight of berries from one raceme in average was 40.68±3.27g. A positive correlation was found between the length of raceme and the weight of berries per raceme. American pokeweed seeds are black, reniform-orbicular, shining, smooth (fig.1a). The size of harvested seeds in season of 2019 was 2.78±0.09cm of length and 1.80±0.20cm of width. Seeds weight was 6.45±0.02g/1000 seeds. Therefore, determined morpho-biological features of *Phytolacca americana* L. plants reproduced from seeds and grown in season of 2019 under the pedoclimatic conditions of the Republic of Moldova did not differ from similar data reported by other scientists (Balogh and Juhasz, 2008; Dzadzieva, 2011). The antioxidant activity of extracts from *Phytolacca americana* L. fruits is depending on extraction procedures and content of polyphenolics (Brindza *et al.,* 2014; Ivanova, 2019). The alcoholic and hydroalcoholic extracts from American pokeweed fruits contained 5.80±0.62mg and 8.33±0.42mg of total polyphenols per g of dry residue, correspondingly. The biggest amount of polyphenolic substances was extracted by water, in aqueous extracts their content was equal 17.63±0.61mg/g. However, it was reported (Nabavi *et al.,* 2009; Zheleva-Dimitrova, 2013) that in extracts from pokeweed fruits obtained by percolation using methanol the content of polyphenolic substances could reached to 102.11±6.37mg equivalent to gallic acid or 174.76±0.74 mg pyrogallol equivalent per g dry extract.

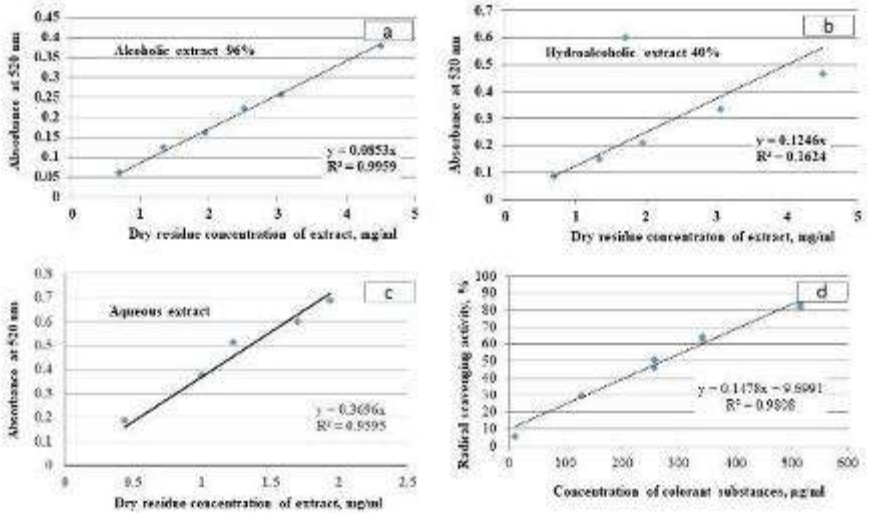

Figure 2. Dependence of absorbance on dry residue concentration of alcoholic (a), hydroalcoholic (b) and aqueous (c) extracts and dose-activity curve of colorant substances (d).

Extraction procedures have also had a major impact on content of colorant substances in fruits extracts (fig. 2 a, b, c). The dependences of extracts absorbance regarding to colorant substances on concentration of dry residue in limits from zero to 4.5 mg/ml were linear with a good approximation (0.9595-0.9959). The absorbance values of different extracts were compared regarding to the same concentration of dry residue, as follows: the absorbance of aqueous extract with concentration 2mg/ml of dry residue was equal 0.7; hydroalcoholic and alcoholic extracts – 0.2 and 0.16, respectively. Thus, aqueous extracts contained 3.5-4.3 times more colorant substances than hydroalcoholic and alcoholic extracts.

The relationship between content of colorant substances and antioxidant activity of fruits extracts was determined (fig.2 d). The colorant substances from American pokeweed fruits exhibited the high radical scavenging activity against peroxyl radicals; IC_{50} was equal to 259.65±2.60µg/ml. It should be noted that the reported data on radical scavenging activity of integrated extracts from American pokeweed fruits against DPPH free radicals differ considerable from IC_{50}=62.0±2.1 µg/ml (Nabavi *et al.*, 2009) to IC_{50}=412.06µg/ml (Zheleva-Dimitrova, 2013). I was determined that the antioxidant activity of natural colorant from American pokeweed fruits was stable during 12 months of storage (Mchedlishvili *et al*., 2014). In our research the antioxidant activity of fruits extract was in direct dependences on content of both polyphenols (R^2=0.9563) and colorant substances (R^2=0.9808). Thus, the American pokeweed fruits contain the chemical constituents such as polyphenolic and colorant compounds, which directly influence on their biological activity.

CONCLUSION

The growth particularities of *Phytolacca americana* L. plants in pedoclimatic conditions of the Republic of Moldova were studied. American pokeweed demonstrated the excellent adaptivity of seeds and seedlings under different condition of germination, transplantation and cultivation. Taking into account the abundant growth of these perennial plants by seasonal renewal and the accumulation of valuable chemical constituents with biological activities, we could recommend to initiate the introduction of *Phytolacca americana* L. into the culture. The polyphenolic and colorant substances from American pokeweed fruits exhibit the high radical scavenging activity and can be used as ingredients in food technologies.

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